

A Golden Gate Modular Cloning Toolbox for Plants

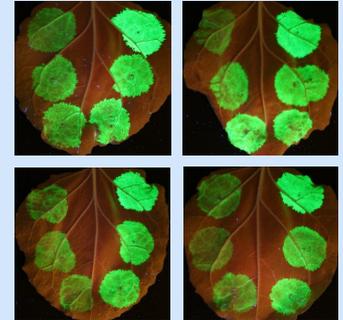


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Plant Synthetic Biology requires robust and efficient methods for assembling multigene constructs. Golden Gate cloning provides a precision module-based cloning technique for facile assembly of multiple genes in one construct. We present a versatile resource for plant biologists comprising a set of cloning vectors and 96 standardised and tested parts to enable Golden Gate construction of multigene constructs for plant transformation. Parts include promoters, untranslated sequences, reporters, antigenic tags, localization signals, selectable markers, and terminators.



Golden Gate cloning is a DNA assembly method based on the use of type IIS restriction enzymes (**Figure 1**). A Modular Cloning (MoClo) assembly standard has been developed in which specified, standard overhangs are used for predefined-modules of basic genetic grammar (**Figure 2**). Standard parts (Level 0) can be assembled into transcriptional units (Level 1) in a single step and, in a second single-step reaction, multiple transcriptional units can be assembled into multi-gene constructs (Levels 2, M and P) (**Figure 3**).

We have published¹ two kits for plant biologists: The ‘Golden Gate Modular Cloning (MoClo) Plant Parts Kit’ comprises 96 tested, standard parts for plants, including promoters, untranslated sequences, reporters, antigenic tags, localization signals, selectable markers, and terminators (*listed in appendix overleaf*).

The ‘Golden Gate MoClo Plant Tool Kit’ contains all the vector backbones and sequences required for domestication of new sequences and assembly into single and multigene binary constructs.

Both kits are available from AddGene <https://www.addgene.org/>

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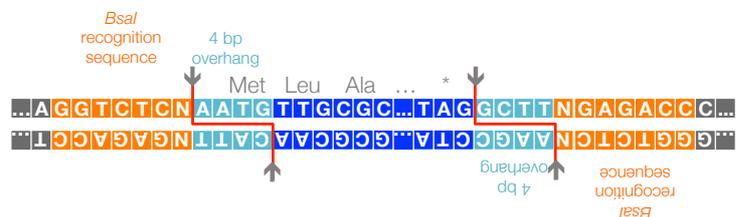


Figure 1. The cleavage sites of the Type IIS enzymes used in Golden Gate cloning are downstream of the recognition site and can be composed of any sequence. After ligation, no scar will exist between adjacent fragments.

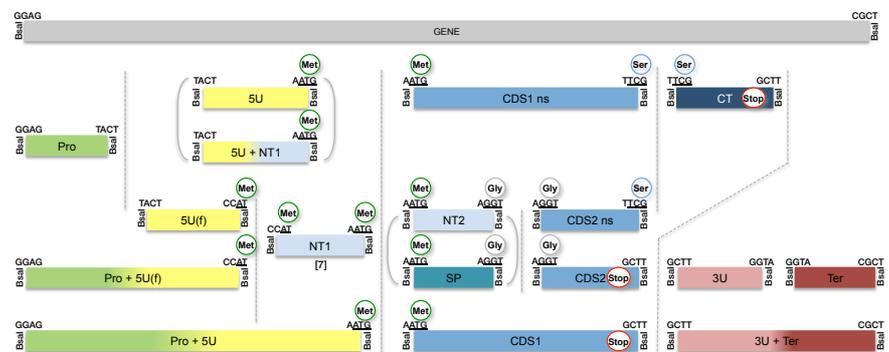


Figure 2. A modular version of Golden Gate Cloning (MoClo) has been developed in which specified, standard overhangs are used for predefined-parts of basic genetic grammar. This simplifies the rules for assembly and allows laboratories to exchange standard modules.

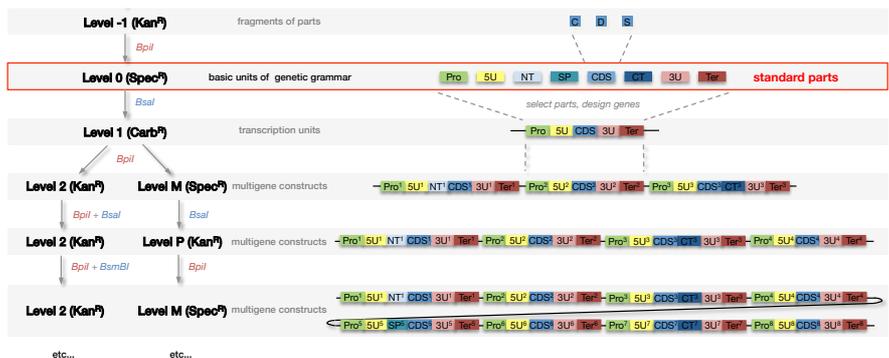


Figure 3. The Golden Gate MoClo Assembly Standard: Standard (Level 0) parts are assembled from single or multiple sequences either directly or via intermediate (Level –1) fragments. Level 0 parts are assembled into Level 1 acceptor backbones to make complete transcriptional units. Multigene constructs can be made by assembling level one constructs in Level 2, M, or P acceptor backbones.

Appendix: The Golden Gate MoClo Plant Parts Kit contains 96 standard parts:

	1	2	3	4	5	6	7	8	9	10	11	12
A	pICH41373 plant virus Pro	pICH45180 plant Pro	pICH51277 plant + virus Pro + 5U	pICH45234 plant Pro + 5U	pICH87611 plant + virus Pro + 5U	pICH78133 chloroplast 5U + NT1	pICSL30008 antigenic NT1	pICSL50007 antigenic CT	pICSL50004 reporter CT	pICH49477 reporter CDS1	pICSL80016 reporter CDS1 ns	pICH44300 plant 3U + Ter
B	pICH41388 CaMV 35s plant virus Pro	pICH45125 plant Pro	pICH51288 plant + virus Pro + 5U	pICH45244 plant Pro + 5U	pICH41402 plant virus 5U	pICH78141 SP 5U + NT1	pICSL30009 antigenic NT1	pICSL50009 antigenic CT	pICSL50011 reporter CT	pICSL80004 reporter CDS1	pICSL70002 selection gene	pICH77901 bacterial 3U + Ter
C	pICH45089 plant virus Pro	pICH45131 plant Pro	pICSL12006 plant virus Pro + 5U	pICH45214 plant Pro + 5U	pICH44199 plant virus 5U	pAGM5331 nuclear TP 5U + NT1	pICSL30004 reporter NT1	pICSL50010 antigenic CT	pICSL50015 reporter CT	pICSL80001 reporter CDS1	pICSL70004 selection gene	pICH77911 bacterial 3U + Ter
D	pICH42211 bacterial Pro	pICH45152 plant Pro	pICH87633 bacterial + virus Pro + 5U	pICH87655 plant + virus Pro + 5U	pICH44222 plant virus 5U	pAGM1482 mitochondrial TP 5U + NT1	pICSL30006 reporter NT1	pICSL50012 antigenic CT	pICSL50006 reporter CT	pICH75111 reporter CDS1	pICS70005 selection gene	pICH41432 bacterial 3U + Ter
E	pICH50581 plant Pro	pICH41551 plant Pro	pICH85281 bacterial Pro + 5U	pICH71292 plant Pro + 5U	pICH44233 plant virus 5U	pAGM5355 chloroplast TP + HIS tag 5U + NT1	pICSL30003 reporter NT1	pICSL50013 antigenic CT	pICSL80014 reporter CDS1	pICH42222 selection CDS1	pICSL70008 selection gene	pICH71431 plant 3U + Ter
F	pICH42760 plant Pro	pICSL13001 plant + virus Pro + 5U(f)	pICH88103 bacterial Pro + 5U	pICH71301 plant Pro + 5U	pICH44188 plant virus 5U	pAGM5343 SP + HIS tag 5U + NT1	pICSL30010 reporter NT1	pICL50014 antigenic CT	pICH41531 reporter CDS1	pICH43844 selection CDS1	pICH41414 plant virus 3U + Ter	pICH71411 plant 3U + Ter
G	pICH44157 plant Pro	pICSL13002 plant + virus Pro + 5U(f)	pICH87644 plant + virus Pro + 5U	pICH71311 plant Pro + 5U	pICH44179 plant 5U	pAGM1467 HIS tag + EK 5U + NT1	pICH37431 signal peptide SP	pICSL50008 reporter CT	pICSL80005 reporter CDS1	pICH44022 silencing suppressor CDS1	pICH72400 bacterial 3U + Ter	pICH71421 plant 3U + Ter
H	pICH45173 plant Pro	pICH51266 plant + virus Pro + 5U	pICH45195 plant Pro + 5U	pICH71342 plant Pro + 5U	pAGM1479 HIS tag 5U + NT1	pICSL30005 antigenic NT1	pICH37326 signal peptide SP	pICSL50016 reporter CT	pICSL80007 reporter CDS1	pICSL80012 reporter CDS2	pICH41421 bacterial 3U + Ter	pAGT707 plant virus 5U(f)

Vector	Part
Promoters (PRO GGAG-TACT)	
A1	pICH41373 1.3 kb 35s (CaMV)
B1	pICH41388 0.4 kb 35s (CaMV)
C1	pICH45089 double 35s (CaMV)
D1	pICH42211 nos (<i>A. tumefaciens</i>)
E1	pICH50581 act2 (AT3G18780, <i>A. thaliana</i>)
F1	pICH42760 spm (<i>Z. mays</i>)
G1	pICH44157 <i>RbcS2B</i> (AT5g38420, <i>A. thaliana</i>)
H1	pICH45173 <i>RbcS1B</i> (AT5g38430, <i>A. thaliana</i>)
A2	pICH45180 <i>RbcS3B</i> (AT5g38410, <i>A. thaliana</i>)
B2	pICH45125 <i>LHB1B1</i> (AT2g34430, <i>A. thaliana</i>)
C2	pICH45131 <i>LHB1B2</i> (AT2g34420, <i>A. thaliana</i>)
D2	pICH45152 <i>cab1</i> (AT1g29930, <i>A. thaliana</i>)
E2	pICH41551 <i>STLS</i> (<i>Solanum tuberosum</i>)
Promoter + 5' UTR (PRO + 5U (f) GGAG-CCAT)	
F2	pICSL13001 1.3 kb + 5'UTR, 35s (CaMV)
G2	pICSL13002 0.4 kb + 5'UTR, 35s (CaMV)
Promoter + 5' UTR (PRO + 5U GGAG-AATG)	
H2	pICH51266 1.3 kb 35s (CaMV) + Ω (TMV)
A3	pICH51277 0.4 kb, 35s (CaMV) + Ω (TMV)
B3	pICH51288 double 35s (CaMV) + Ω (TMV)
C3	pICSL12006 Cassava Vein Mosaic Virus
D3	pICH87633 Nos (<i>A. tumefaciens</i>) + Ω (TMV)
E3	pICH85281 Mas (<i>A. tumefaciens</i>) + Ω (TMV)
F3	pICH88103 Ocs (<i>A. tumefaciens</i>) + Ω (TMV)
G3	pICH87644 Act2 (AT3G18780, <i>A. thaliana</i>) + Ω (TMV)
H3	pICH45195 <i>RbcS2B</i> (AT5g38420, <i>A. thaliana</i>)
A4	pICH45234 <i>RbcS2B</i> (AT5g38430, <i>A. thaliana</i>)
B4	pICH45244 <i>RbcS3B</i> (AT5g38410, <i>A. thaliana</i>)
C4	pICH45214 <i>LHB1B1</i> (AT2g34430, <i>A. thaliana</i>)
D4	pICH87655 <i>cab2</i> (AT1g29930, <i>A. thaliana</i>) + Ω (TMV)
E4	pICH71292 <i>RbcS2</i> (<i>S. lycopersicum</i>)
F4	pICH71301 <i>RbcS2</i> (<i>S. lycopersicum</i>)
G4	pICH71311 <i>RbcS3A</i> (<i>S. lycopersicum</i>)
H4	pICH71342 <i>H4</i> (<i>S. lycopersicum</i>)
A5	pICH87611 <i>STLS</i> (<i>S. tuberosum</i>) + Ω (TMV)
5' UTR (5U TACT-AATG)	
B5	pICH41402 Ω (TMV)
C5	pICH44199 Ω (Potato Virus X)
D5	pICH44222 CMV1 (Cucumber Mosaic Virus)
E5	pICH44233 CMV2 (Cucumber Mosaic Virus)
F5	pICH44188 BSMV (Barley Stripe Mosaic Virus)
G5	pICH44179 <i>RbcS2B</i> (AT5g38420, <i>A. thaliana</i>)
5' UTR (5U(f) TACT-CCAT)	
H12	pAGT707 H12
5' UTR + tag/leader (5U+NT1/SP TACT-AATG)	
H5	pAGM1479 Ω (TMV) + HIS tag (6x polyhistidine)
A6	pICH78133 Ω (TMV) + chloroplast transit peptide <i>RbcS</i> (synthetic)
B6	pICH78141 Ω (TMV) + signal peptide RAm3A (<i>Oryza sativa</i>)
C6	pAGM5331 5'UTR, Ω (TMV) + nuclear localisation signal (Simian Virus 40)
D6	pAGM1482 5'UTR, Ω (TMV) + mitochondrial localisation signal <i>ScCoxIV</i> (<i>Saccharomyces cerevisiae</i>)
E6	pAGM5355 5'UTR, Ω (TMV) + chloroplast transit peptide, <i>RbcS</i> (synthetic) + HIS tag
F6	pAGM5343 5'UTR, Ω (TMV) + signal peptide, RAm3A (<i>O. sativa</i>) + HIS tag
G6	pAGM1467 5'UTR, Ω (TMV) + HIS tag + enterokinase (EK) cleavage site

Vector	Part
N terminal tag (NT1 CCAT-AATG)	
H6	pICSL30005 3x FLAG tag
A7	pICSL30008 6x HA tag
B7	pICSL30009 4x Myc tag
C7	pICSL30004 YFP variant of GFP (<i>A. victoria</i>)
D7	pICSL30006 GFP (<i>A. victoria</i>)
E7	pICSL30003 mCherry (<i>Discosoma</i> sp.)
F7	pICSL30010 mEOS2 (<i>Lobophyllia hemprichii</i>)
Signal Peptide (SP AATG-AGGT)	
G7	pICH37431 RAm3A (<i>O. sativa</i>)
H7	pICH37326 CRT (<i>N. benthamiana</i>)
C-Terminal Tag (CT TTCG-GCTT)	
A8	pICSL50007 3x FLAG tag
B8	pICSL50009 6x HA tag
C8	pICSL50010 4x Myc tag
D8	pICSL50012 V5 tag (Simian Virus 5)
E8	pICSL50013 T7 tag (bacteriophage T7 gene 10)
F8	pICSL50014 HBP tag (HA-PreScission-Biotin)
G8	pICSL50008 GFP (<i>A. victoria</i>)
H8	pICSL50016 turboGFP (<i>P. plumata</i>) with U5 intron (<i>A. thaliana</i>)
A9	pICSL50004 mCherry (<i>Discosoma</i> sp.)
B9	pICSL50011 EOS (<i>L. hemprichii</i>)
C9	pICSL50015 mNEON (<i>Branchistoma lanceolatum</i>)
D9	pICSL50006 CDS, luciferase (<i>Photinus pyralis</i>)
Coding Sequence (CDS1 AATG-GCTT)	
E9	pICSL80014 YFP variant of GFP (<i>A. victoria</i>)
F9	pICH41531 GFP (<i>A. victoria</i>)
G9	pICSL80005 turboGFP (<i>Pontellina plumata</i>)
H9	pICSL80007 mCherry (<i>Discosoma</i> sp.)
A10	pICH49477 DsRed (<i>Discosoma</i> sp.)
B10	pICSL80004 turbo RFP (<i>Entacmaea quadricolor</i>)
C10	pICSL80001 luciferase (<i>P. pyralis</i>)
D10	pICH75111 β-glucuronidase (<i>E. coli</i>) with two introns
E10	pICH42222 phosphinothricin acetyl transferase, (<i>S. hygroscopicus</i>)
F10	pICH43844 phosphinothricin acetyl transferase, (<i>S. hygroscopicus</i>) with intron from <i>Act2</i> (<i>A. thaliana</i>)
G10	pICH44022 P19 suppressor of gene silencing (Tomato Bushy Stunt Virus)
Coding Sequence (CDS2 AGGT-GCTT)	
H10	pICSL80012 YFP (<i>A. victoria</i>)
Coding Sequence no stop (CDS1 ns AATG-TTCG)	
A11	pICSL80016 β-glucuronidase (<i>E. coli</i>) with two introns
Transcriptional Unit (GGAG-CGCT)	
B11	pICSL70002 BASTA selection cassette
C11	pICSL70004 kanamycin selection cassette
D11	pICSL70005 bialaphos selection cassette
E11	pICSL70008 pFAST-R selection cassette
3' UTR + Terminator (3U + Ter GCTT-CGCT)	
F11	pICH41414 35s (CaMV)
G11	pICH72400 <i>g7</i> (<i>A. tumefaciens</i>)
H11	pICH41421 <i>nos</i> (<i>A. tumefaciens</i>)
A12	pICH44300 <i>act2</i> (<i>A. thaliana</i>)
B12	pICH77901 <i>mas</i> (<i>A. tumefaciens</i>)
C12	pICH77911 <i>ags</i> (<i>A. tumefaciens</i>)
D12	pICH41432 <i>ocs</i> (<i>A. tumefaciens</i>)
E12	pICH71431 <i>ATPase</i> (<i>S. lycopersicum</i>)
F12	pICH71411 <i>RbcS3C</i> (<i>S. lycopersicum</i>)
G12	pICH71421 Histone H4 (<i>S. tuberosum</i>)

The Golden Gate MoClo Plant Tool Kit (not shown) contains all the vector backbones and sequences required for domestication of new sequences and assembly into single and multigene binary constructs. The Golden Gate MoClo Plant Parts Kit (above and left) contains 96 standard parts.

To confirm function and compare strength, parts of each type were cloned into equivalent GFP expression cassettes and tested in *Nicotiana benthamiana* leaves by *Agrobacterium*-mediated transfection.

The example below compares 21 x 'Promoter + 5' UTR' parts. Data for all parts are given in Supplementary Data 6-10 of Engler et al. (2014) ACS Synthetic Biology DOI: 10.1021/sb4001504

